Brain, Behavior, and Immunity 26 (2012) 597–606

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Immunological parameters in elderly women: Correlations with aerobic power, muscle strength and mood state

Vagner Raso [a,b,c,*], Valéria Maria Natale [a,d], Alberto José da Silva Duarte [c], Júlia Maria D'Andrea Greve [a,b], Roy J. Shephard [e]

[a] Department of Experimental Pathophysiology, Medical School of the University of Sao Paulo, FMUSP, Brazil
[b] Movement Studies Laboratory, Orthopaedics and Traumatology Institute, Clinics Hospital, Medical School of the University of Sao Paulo, LEM-IOT-HC-FMUSP, Brazil
[c] Laboratory of Investigation in Dermatology and Immunodeficiencies, Medical School of the University of Sao Paulo, FMUSP, Brazil
[d] Clinics Hospital, Medical School of the University of Sao Paulo, HC-FMUSP, Brazil
[e] Faculty of Physical Education and Health, University of Toronto, Toronto, ON, Canada

ARTICLE INFO

Article history:
Received 22 September 2011
Received in revised form 23 December 2011
Accepted 18 January 2012
Available online 28 January 2012

Keywords:
Aerobic power
Aging
Depression
Fatigue
Immune system

ABSTRACT

Purpose: Our objective was to relate immunological data for healthy but sedentary elderly women to aerobic power, strength, and mood state.

Methods: We measured peak aerobic power and one-repetition maximum strength along with mood (depression and fatigue), quality of life and carbohydrate intake on 42 women aged 60–77 years. Standard immunological techniques determined natural killer cell count and cytotoxic activity (NKCA), proliferative responses to phytohemaglutinin and OKT_3, various lymphocyte subpopulations ($CD3^+$, $CD3^-CD19^+$, $CD56^+$, $CD4^+$, $CD8^+$, $CD56^{dim}$ and $CD56^{bright}$), and markers of activation, maturation, down-regulation and susceptibility to apoptosis ($CD25^+$, $CD28^+$, $CD45RA^+$, $CD45RO^+$, $CD69^+$, $CD95^+$, HLA-DR^+).

Results: Correlations of immune parameters with aerobic power and strength were very similar for absolute and relative immunological data. In the group as a whole, the only correlation with aerobic power was −0.35 (relative $CD4^+CD69^+$ count), but in subjects with values <22.6 mL kg^{-1} min^{-1} correlations ranged from [illegible] to +0.48 (absolute [illegible] cell proliferation were correlated with aerobic power or muscle strength. Although mood state and quality of life can sometimes be influenced by an individual's fitness level, our multivariate analyses suggested that depression, fatigue and quality of life were more important determinants of immune profile than our fitness measures.

Conclusions: Psychological changes associated with aging may have a substantial adverse effect upon the immune system, and immunological function may be enhanced more by addressing these issues than by focusing upon aerobic or resistance training.

1. Introduction

Aging is associated with a decrease in the efficacy of vaccines and a progressive increase in the prevalence of infections (Grubeck-Loebenstein et al., 2009; Targonski et al., 2007). These changes reflect in part poor nutrition, the cumulative effects of cigarette smoking and exposure to air pollutants, a progressive breakdown of muco-cutaneous barriers, a depression of mood state and an accumulation of various chronic pathologies (Shephard, 1997). One study argued that the immune system was not necessarily compromised even in individuals who reached 100 years of age (Strindhall et al., 2007), but other investigators have pointed to deteriorations in several specific aspects of immune function, including a decline in T cell function (Ginaldi et al., 1999; Makinodan et al., 1991; Pawelec et al., 2002), decreased pools of naive T and B cells, increases in the number of memory and effector T and B cells, an accumulation of late differentiated effector T cells, and a diminished B cell production of immunoglobulins secondary to a reduced activity of T helper lymphocytes (Ben Yehuda and Weksler, 1992; Antonaci et al., 1987). Generally, there is an increase in $CD56^{dim}$ counts, with a decrease in the overall number and/or activity of NK cells, and a decreased affinity for target cells (Grubeck-Loebenstein et al., 2009; Nasrullah and Mazzeo, 1992), particularly in unfit subjects (Ross et al., 2004).

It is less clear how far an age-related decrease in maximal aerobic power and/or muscle strength accounts for impairments of immune

* Corresponding author at: Movement Studies Laboratory, Orthopaedics and Traumatology Institute, #333 Dr. Ovídio Pires de Campos street, 2nd floor, 05403-010 Sao Paulo, SP, Brazil. Tel./fax: +55 11 3069 6041.
E-mail address: vraso@usp.br (V. Raso).

0889-1591 © 2012 Elsevier Inc. Open access under the Elsevier OA license.
doi:10.1016/j.bbi.2012.01.012

function, and it remains uncertain whether the immune handicaps of the elderly can be made good by a regular aerobic or resistance training programme. Shinkai et al. (1998) made cross-sectional comparisons between 65-year-old elite distance runners and their sedentary peers; comparing non-smokers in the two groups, they saw little inter-group difference in $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$ or $CD19^+$ counts; the runners did show a superior T cell proliferative response to both phytohemagglutinin (PHA), and pokeweed mitogen, but the mixed lymphocyte reaction was not enhanced, making it unlikely that the runners had a better T cell effector function. Nieman et al. (1993) also made a cross-sectional comparison between fit and unfit women aged 67–85 years; in their study, the trained individuals had a 54% advantage of lytic activity and a 56% greater T cell proliferative response to PHA, but there were no inter-group differences in lymphocyte subset counts; moreover, a 12-week programme of moderate aerobic exercise did not enhance either T cell function or resting NK cell activity in the sedentary group. In contrast, Crist et al. (1989) were able to induce a 33% increase in resting NK cell activity in seven elderly subjects following 16 weeks of vigorous aerobic training. Bruunsgaard and Pedersen (2000) concluded that although highly conditioned individuals seem to have a relatively better preserved immune system, it is unclear whether this advantage is linked to their training or to other lifestyle-related factors.

The objectives of this study were thus to report phenotypic and functional immunological parameters in a substantial sample of relatively sedentary but otherwise healthy elderly women carefully screened for other factors that might adversely affect their immune function, and to examine relationships between the immunological findings, aerobic power, muscle strength and mood state.

2. Methods

2.1. Subjects

A convenience sample of 73 sedentary but otherwise healthy female volunteers aged 60–77 years was recruited from the community of Sao Paulo, Brasil. They were informed about the procedures and risks before giving their written consent to participation in a study approved by the research ethics committee of the University of Sao Paulo Medical School. A preliminary telephone screening that focused on current health status, drug and cigarette use, and habitual physical activity was followed by a hospital visit for a detailed history and physical examination covering past and current health status, symptoms of depression, self-reported ability to perform the basic and instrumental activities of daily living, a 12-lead electrocardiogram, an assessment of body composition, and general laboratory blood and urine tests according to the SENIEUR protocol. Thirty-one of the initial 73 volunteers were excluded for factors that could have modified their immune function: (i) participation in a regular physical activity programme during the previous three months; (ii) involvement in alternative dietary therapy; (iii) undernourishment or obesity, (iv) cigarette smoking; (v) cardiovascular, pulmonary, or metabolic disease, chronic infectious or auto-immune disease; (vi) central or peripheral nervous system disorders; (vii) treatment for, or a history of cancer; (viii) chronic use of corticosteroids; (ix) any kind of surgery during the previous three months; (x) forced bed rest during the previous three months; and (xi) any orthopedic conditions that could limit exercise or be exacerbated by exercise testing.

2.2. Carbohydrate intake

Volunteers self-recorded their eating habits during three typical days (two week days and one weekend day). The estimate of carbohydrate intake represents the mean of records for the three days.

2.3. Depression and fatigue

Volunteers completed the profile of mood states questionnaire (POMS) with respect to the last week, and scores were calculated for depression/dejection and fatigue/inertia (McNair and Droppleman, 1971); potential values ranged from 0 to 60 for depression/dejection, and from 0 to 28 for fatigue/inertia, with high values indicating an unfavourable score.

2.4. Quality of life

The individual's perceived quality of life (QOL) during the past two weeks was assessed using the short questionnaire proposed by the World Health Organization (WHO) (Skevington et al., 2004). Volunteers evaluated each item in four domains (physical, psychological, social-relational, and environmental), using a five-point Likert scale and scoring from 1 (very dissatisfied/very poor) to 5 (very satisfied/very good). Summing across these four domains, we calculated an overall quality of life; with a potential score ranging from 24 to 120, and a high number indicating a good quality of life.

2.5. Aerobic power

The peak aerobic power ($\dot{V}O_{2peak}$) was measured using a modified Bruce treadmill test protocol (American College of Sports Medicine, 2006). Subjects walked on an ATL-10200 treadmill (Inbramed, Porto Alegre, RS, BRA) with continuous monitoring of a 12-lead electrocardiogram, blood pressure, and metabolic response (CPX/D metabolic cart, Medgraphics, St Paul, MN, calibrated by gases of known composition immediately before each stress test). After collecting three minutes of resting data with the subject standing on the treadmill, walking began at 2.6 km h^{-1}, 5% grade, and thereafter the speed and grade were increased every three minutes to volitional fatigue. Criteria of $\dot{V}O_{2peak}$ were: (i) RER > 1.10; (ii) attainment of maximal age-predicted heart rate; and (iii) volitional fatigue.

2.6. Muscle strength

Muscle strength was determined as the one repetition maximal (1RM) effort attained in a leg press exercise; it reflected the maximum load (N) that a subject could lift just once, using the required technique (applying the force via the specified muscle groups, without assistance from momentum or changes in body position). Three familiarization sessions each comprised three sets of eight to 12 repetitions of the leg press exercise preceded the definitive test.

2.7. Blood collection and white blood cell counts

Subjects avoided solid or liquid foods containing caffeine, chocolate, or cola-based products, and moderate or vigorous physical activity for 48 h prior to collection of blood samples. They came to the laboratory at 7:00 a.m., having fasted overnight, and antecubital blood samples were collected after 30 min of seated rest. Blood in non-heparinized syringes was dispensed into evacuated tubes coated with ethylene diamine tetra-acetic acid (EDTA) and kept refrigerated until analysis later on the same day, when differential cell counts were made using a Cell-Dyn 3500 cell analysis system (Coulter Corp., Miami, FL). Proliferative responses and natural killer cell activity (NKCA) were tested on samples collected in heparinized syringes after an interval of no more than 4 h.

2.8. Flow cytometry

Two hundred microliters of whole blood was incubated for one-, two-, or three-color immunophenotyping, using appropriate

combinations of monoclonal antibodies (Becton–Dickinson, Miami, FL) conjugated to fluorescein isothiocyanate (FITC (CD25, CD45RA, CD95)), phycoerythrin (PE (CD19, CD28, CD45RO, CD69, HLA-DR)), or phycoerythrin-cyanine (PE-Cy-5 or PCy-5 (CD3, CD4, CD8, CD56)). The conjugated monoclonal antibodies (MAb) used were CD3(PCy-5)/CD4(FITC)/CD8(PE) and CD3(FITC)CD19(PE). $CD56^{dim}$ and $CD56^{bright}$ cells were distinguished by appropriate gating in the $CD56^{+}$ region. Whole-blood aliquots with appropriate MAbs were incubated in the dark at room temperature for 20 min. Samples with isotypic control antibodies (IgG1[FITC]/IgG1[PE]/IgG1[PCy-5) were run in parallel with each sample. A minimum of 5000 cells was analyzed on a Coulter XL-MCL (Coulter Corp., Miami, FL), and data analyses were performed using XL System II software. Lymphocyte analyses were performed by gating on the lymphocyte region, based on forward and side light scatter. Counts for each subset were obtained by multiplying the total lymphocyte count by the percentage of the respective subset.

2.9. Peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. They were then diluted in RPMI (GIBCO, Carlsbad, CA) with 5% heat-inactivated fetal calf serum (FCS, Sigma–Aldrich), gentamicin (40 μg mL^{-1}), glutamine (200 mM), and 2-mercaptoethanol (5×10^{-5} M) (complete medium).

2.10. Lymphocyte proliferative response

The lymphocyte proliferative response was measured by ^{3}H-thymidine incorporation after stimulation by phytohemaglutinin (PHA) or muromonabCD3 (OKT3, Janssen, Beerse, Belgium). The freshly isolated PBMC were adjusted to 2×10^{6} cells per milliliter, and 100 μL of the suspension was plated in triplicate wells of a 96-well, round-bottomed microplate (Costar, Cambridge, MA). PHA or OKT3 was diluted to a final concentration of 5 μg mL^{-1}. The plates were incubated at 37 °C for 72 h in an atmosphere of 5% CO_2 and were then pulsed with 1 μCi per well of ^{3}H-thymidine (6.7 Ci·$mmol^{-1}$, ICN Biomedicals, Irvine, CA), 18 h before harvesting onto glass-fiber filter paper (Skatron Cell Harvester, Norway). Five milliliters of scintillation fluid were added to the filters, and they were counted in a β-plate scintillation counter (Wallac Oi, Turku, Finland). The control count was subtracted from the mitogenic count and values were expressed as counts per minute.

2.11. Natural killer cell cytotoxic activity

Natural killer cell cytotoxic activity (NKCA) was measured using the standard NK-sensitive K562 cell line and a radioactive chromium release assay. The human erythromyeloid leukemia-derived cell line K562 was maintained in RPMI 1640, supplemented with 10% FBS, gentamicin (40 μg mL^{-1}), and Hepes buffer (Sigma–Aldrich, São Paulo), kept in 5% CO_2 at 37 °C. Freshly isolated Ficoll-purified PBMC were adjusted to 1×10^{7} cells per milliliter in complete medium and were then diluted serially at 40:1, 20:1, 10:1, and 5:1 effector-to-target (E:T) ratios. The PBMC were placed into 96-well round-bottom microtiter plates and incubated with radiolabeled K562 cells. K562 cells were labeled with 100 μCi·10^{-6} cells of sodium ^{51}chromate (^{51}Cr; ICN Biomedicals, Irvine, CA) over a 1-h period in a shaking waterbath at 37 °C. After a further 4 h of incubation at 37 °C and 5% CO_2, the plates were centrifuged at 100 g for 5 min. The supernatant (100 μL) was transferred to polypropylene tubes, and the released radioactivity was counted in a gamma counter (1275 minigamma, LKB-Wallac, Turku, Finland). The total release of chromium was determined in wells containing ^{51}Cr-labeled cells with RPMI 1640, 10% FBS with 10% triton X-100. Spontaneous release was always less than 10% of total release. NKCA was calculated as the mean of triplicate determinations for each E:T ratio and was expressed as percentage lysis, calculated as follows:

$$\%\text{lysis} = \frac{\text{mean experimental counts per minute} - \text{mean spontaneous counts per minute}}{\text{mean maximum counts per minute} - \text{mean spontaneous counts per minute}} \times 100$$

2.12. Statistical analysis

The necessary sample size for our observations was calculated using SigmaStat software (Jandel Scientific, San Rafael, CA), as described previously (Raso et al., 2007), with $\alpha = 0.05$ and $\beta = 0.20$. A one-sample Kolmogorov–Smirnov test demonstrated the normality of data distribution for all measured variables. Basic data are presented as means ± standard error of the mean. Independent sample "*t*" tests compared subjects grouped according to their fitness percentile (i.e., $P_0 - P_{50}$ *versus* $P_{50} - P_{100}$) for aerobic power and muscle strength. Univariate and hierarchical multiple regression analysis investigated associations of phenotypic and functional immunological parameters with aerobic power, muscle strength and mood state. Bonferroni corrections were applied where appropriate. All analyses were performed using Predictive Analytics Software 17.0 for Windows package (PASW, Inc., Chicago, IL).

3. Results

3.1. General physical characteristics

With few exceptions, subjects fell into the "young-old" age category. Scores for the various measures of fitness, mood state and carbohydrate intake were all at the levels anticipated for relatively inactive but otherwise healthy individuals in this age category (Table 1). The average body mass index was only a little above the ideal range, and the average participant was obtaining <40% of the estimated total energy intake of 6.90 ± 0.34 MJ day^{-1}; 1659 ± 81 kcal day^{-1} from carbohydrate; however, there were wide inter-individual differences, probably due in part to imprecise reporting and some under-reporting of overall food consumption.

When aerobic power values were used to classify subjects into upper and lower halves of a fitness continuum, fitter subjects had a lower BMI ($P = .033$), body fat content ($P = .001$), and muscle strength ($P = .041$) (Table 1). However, there were no significant differences of general physical characteristics when subjects were categorized in terms of muscle strength. Scores for the psychobiological variables (depression, fatigue and quality of life) were not significantly influenced by either measure of fitness.

3.2. Immune parameters

Values for a wide range of immune parameters are summarized in Table 2, with arrows indicating the anticipated trend of older individuals relative to published values for young women. Where precise data are available, and with the exception of values for the NK cells, our results generally fall within the anticipated range for young adults.

3.3. Effects of age

Despite the relatively small age range among our subjects, univariate regression coefficients were at or near statistical significance for several immune variables (Table 3), with the absolute values for

Table 1
General characteristics of subjects considered as a whole and when assigned to upper and lower halves of a fitness continuum in terms of their using aerobic power and muscle strength (mean ± SEM, range).

	Aerobic power (mL kg^{-1} min^{-1})		Muscle strength (N)		Total
	<22.6	≥22.6	<750	≥750	
Age (years-old)	69.1 ± 1.1 (62.0–77.0)	66.9 ± 0.9 (61.0–73.0)	68.7 ± 1.2 (61.0–77.0)	67.5 ± 0.9 (61.0–77.0)	67.9 ± 0.7 (61.0–77.0)
Height (m)	1.56 ± 0.02 (1.43–1.71)	1.56 ± 0.01 (1.48–1.64)	1.54 ± 0.02 (1.43–1.65)	1.56 ± 0.1 (1.46–1.71)	1.56 ± 0.01 (1.43–1.71)
Body mass (kg)	64.9 ± 3.0 (49.2–96.9)	58.3 ± 1.9 (43.8–81.7)	59.6 ± 3.3 (43.8–96.9)	63.1 ± 2.3 (49.2–81.7)	61.9 ± 1.8 (43.8–96.9)
BMI (kg m^2)	26.7 ± 1.0 (20.8–35.6)	24.0 ± 0.7 (18.4–30.4)*	25.2 ± 1.2 (18.4–35.6)	25.8 ± 0.8 (20.7–34.9)	25.5 ± 0.7 (18.4–35.6)
LBM (kg)	42.7 ± 1.5 (35.4–55.2)	42.4 ± 1.3 (36.0–57.5)	41.0 ± 1.4 (36.0–55.2)	43.5 ± 1.3 (35.4–57.5)	42.6 ± 0.9 (35.4–57.5)
Fat (%)	34.1 ± 1.2 (26.0–43.0)	28.3 ± 1.0 (18.0–37.0)*	30.4 ± 1.7 (18.0–43.0)	32.1 ± 1.1 (25.0–40.0)	31.3 ± 0.9 (18.0–43.0)
AP (mL kg min^{-1})	19.7 ± 0.4 (16.4–22.4)	26.4 ± 1.2 (22.6–45.5)	23.4 ± 1.2 (16.4–33.2)	22.2 ± 0.7 (17.3–28.5)	23.1 ± 0.8 (16.4–45.5)
Muscle strength (N)	77.3 ± 2.1 (60.0–90.0)	71.1 ± 2.0 (50.0–90.0)*	65.7 ± 1.6 (50.0–70.0)	79.8 ± 1.1 (75.0–90.0)	74.1 ± 1.5 (50.0–90.0)
CHO (g·day^{-1})	214.4 ± 17.3 (97.7–345.3)	216.2 ± 16.7 (137.0–371.3)	198.5 ± 19.3 (137.0–371.3)	220.5 ± 14.1 (97.7–345.3)	217.8 ± 11.7 (97.7–371.3)
QOL	95.9 ± 2.1 (73.0–115.0)	96.5 ± 2.6 (70.0–114.0)	94.2 ± 2.8 (70.0–114.0)	96.5 ± 2.2 (73.0–115.0)	96.1 ± 1.6 (70.0–115.0)
Depression	6.3 ± 2.0 (0.0–32.0)	4.7 ± 1.6 (0.0–27.0)	4.3 ± 1.3 (0.0–13.0)	6.2 ± 2.0 (0.0–32.0)	5.4 ± 1.2 (0.0–32.0)
Fatigue	3.2 ± 0.7 (.0–12.0)	2.3 ± 0.6 (0.0–8.0)	3.0 ± 0.7 (0.0–7.0)	2.6 ± 0.7 (0.0–12.0)	2.8 ± 0.4 (0.0–12.0)

BMI: body mass index; LBM: lean body mass; AP: aerobic power; CHO: carbohydrate intake; QOL: quality of life.
* $p < 0.05$;

the CD8$^+$ naïve and memory cells, CD3$^+$ and CD4$^+$ cell activation, and relative values for CD56^{dim} cells all increasing with age.

3.4. Individuals at immunological risk

We observed only three individuals with what clinicians might regard as an adverse immune risk profile (IRP, using as a simple marker of this a CD4:CD8 ratio less than 1.0) (Table 2). Their CD4$^+$ counts were, respectively: 222, 665 and 1058 cells mm^3. These three individuals did not stand out in terms of age or fitness scores when compared with non-IRP subjects, and only one of the three showed elevated scores for depression and fatigue, and a low QOL score. Nevertheless, relative values for these individuals were significantly higher than those for the remainder of our sample in terms of T cell sub-groups (CD3$^+$CD8$^+$, $P = .010$), natural killer cell subtypes (CD56^{dim}CD69$^+$, $P < .0005$), costimulatory molecules and apoptotic markers (CD4$^+$CD95$^+$, $P < .0005$; CD8$^+$CD95$^+$, $P = .001$; CD56^{bright}CD28$^+$, $P = .001$; CD56^{bright}CD95$^+$CD28$^+$, $P < .0001$), naïve and memory cells (CD8$^+$CD45RO$^+$, $P < .0005$; CD4$^+$CD45RA$^+$CD45RO$^+$, $P < .0005$; CD8$^+$CD45RA$^+$CD45RO$^+$, $P = .001$) and T lymphocytes (CD4$^+$HLA-DR$^+$, $P = .022$; CD4$^+$CD25$^+$HLA-DR$^+$, $P = .006$), and were significantly lower for CD3$^+$CD4$^+$ ($P < .0005$), CD4/CD8 ratio ($P < .0005$), CD3$^-$CD19$^+$ ($P = .019$) and CD8$^+$CD45RA$^+$ ($P = .046$). IRP-individuals also showed higher absolute for lymphocytes (CD3$^+$CD8$^+$, $P < .0005$), costimulatory molecules and apoptotic markers (CD56^{dim}CD95$^+$, $P < .0005$; CD56^{bright}CD28$^+$, $P = .010$; CD56^{bright}CD95$^+$, $P < .0005$; CD56^{bright}CD95$^+$CD28$^+$, $P < .0005$), naïve and memory cells (CD4$^+$CD45RA$^+$CD45RO$^+$, $P = .003$; CD8$^+$CD45RA$^+$CD45RO$^+$, $P = .015$), and lower values for CD8$^+$CD45RA$^+$ ($P < .0005$), CD3$^+$CD25$^+$ ($P < .0005$), and lympho-proliferative response (regardless of the stimulus, PHA, $P < .0005$; OKT3, $P = .001$).

3.5. Associations between immune parameters and fitness variables

Counts for lymphocytes, CD3$^+$, CD4$^+$, CD8$^+$, CD3$^-$CD19$^+$, CD3$^-$CD16$^+$CD56$^+$ cells, as well as the expression of CD45RA$^+$, CD45RO$^+$, CD56^{dim}, CD56^{bright}, CD28$^+$, CD95$^+$, CD25$^+$, HLA-DR$^+$, and CD69$^+$ on T lymphocytes and NK sub-types showed no inter-group differences when subjects were classified in terms of aerobic power (<22.6 or >22.6 mL kg^{-1} min^{-1}) or muscle strength (<750 or >750 N). Using this type of classification, there were also no differences in NKCA or lymphocyte proliferation, regardless of the stimulant used (PHA or OKT3) (data not shown).

Univariate correlations of immunological parameters with aerobic power and muscle strength generally showed similar relationships for absolute and relative data (Table 4). Correlations for oxygen intake were seen mainly in the sub-group of women with a lower aerobic power (CD4$^+$CD45RO$^+$, CD56^{dim}CD25$^+$, CD56^{dim}HLA-DR$^+$, CD56^{dim}CD25$^+$HLA-DR$^+$, CD56^{bright}CD25$^+$, CD8$^+$CD95$^+$). Relatively few significant correlations were seen for the group with a high aerobic power (CD4$^+$CD45RA$^+$ [REL: −0.45, $P < .048$], CD3$^+$CD69$^+$ [REL: −0.53, $P < .016$]) and only one negative correlation (with the cell activation marker CD4$^+$CD69$^+$) was significant for the group as a whole. In terms of muscle strength, significant positive correlations were found for several T cell activation markers and memory cell counts: CD3$^+$HLA-DR$^+$, CD3$^+$CD25$^+$HLA-DR$^+$, CD4$^+$CD25$^+$HLA-DR$^+$ and CD8$^+$CD45RA$^+$CD45RO$^+$, although significant relationships were limited to the stronger half of our sample. Data for the group as a whole showed similar (but weaker) positive relationships and also a negative correlation with the CD3$^+$CD4$^+$CD8$^+$ count (Fig. 1). Neither natural killer cell cytotoxic activity nor lymphocyte proliferation data were significantly correlated with either aerobic power or muscle strength (data not shown).

For the purpose of multiple regression analyses, a FITscore was calculated as a half of the sum of [Z aerobic power + Z muscle strength]. Other variables introduced into the equations were the depression, fatigue and quality of life indices and the carbohydrate intake. After appropriate Bonferroni adjustment of probability levels, many of the apparent relationships with the fitness score became non-significant, the only significant items being the numbers of regulatory cells CD3$^+$HLA-DR$^+$ and CD3$^+$CD25$^+$HLA-DR$^+$ (Table 5). The depression score showed a positive association with the relative number of CD3$^+$CD8$^+$ (suppressor) cells, and a negative association with absolute numbers of CD3$^+$CD25$^+$HLA-DR$^+$ regulatory cells. Fatigue scores showed a strong positive association with the numbers of mature CD56^{dim} cells and with the relative numbers of CD4$^+$CD45RO$^+$ memory cells, and a strong negative relationship with PHA proliferation. A good QOL score also showed positive relationships with the relative number of CD3$^+$CD8$^+$ cells and the relative numbers of CD4$^+$CD45RO$^+$ memory cells (i.e. the opposite correlations found for depression), and negative associations with activation markers and PHA proliferative response (i.e. the opposite of the correlation that was found for fatigue). Carbohydrate intake showed only one weak positive association with an activation marker.

Further regression analyses were calculated, testing a series of immune functions against depression, fatigue, QOL, carbohydrate intake and either aerobic power (Table 6a), muscle strength (Table 6b) or FITscore (Table 6c). The only positive correlations with the fitness variables were for CD3$^+$HLA-DR$^+$ (muscle strength and FITscore) and PHA proliferation (FITscore), although several positive relationships were found for depression, fatigue and QOL.

Table 2
Immune parameters for our subjects (mean ± SEM, range) for entire sample, individuals under IRP, and comparison with published norms for young adults (upward arrow indicates value higher in older subjects) shading indicates variables that differ significantly between individuals under IRP and group as a whole.

	IRP individuals	Total sample	Young adults
Lymphocytes			
$CD3^+CD4^+$	648.3 ± 241.7 (221.5–1058.4)	1310.1 ± 89.9 (221.5–2809.4)	500–1300
$CD3^+CD8^+$	1036.3 ± 307.0 (705.7–1649.7)[a]	651.5 ± 44.1 (277.2–1649.7)	300–800
$CD3^+CD4^+CD8^+$	36.1 ± 16.0 (5.5–59.4)	63.8 ± 11.1 (3.1–331.7)	↑
CD4:CD8 ratio	0.6 ± 0.2 (0.3–0.9)	2.1 ± 0.2 (0.3–4.9)	1.5–1.7
$CD3^-CD19^+$	137.4 ± 56.3 (80.6–250.0)	240.0 ± 21.0 (80.6–596.4)	↓ 400
$CD3^-CD16^+CD56^+$	18.3 ± 0.3 (18.0–18.5)	17.0 ± 1.1 (5.5–27.8)	↑ 400–500; 160–400
Naïve and memory cells			
$CD4^+CD45RA^+$	344.1 ± 91.7 (181.9–499.5)	502.9 ± 53.1 (82.1–1533.1)	↓ ?70
$CD8^+CD45RA^+$	469.0 ± 239.8 (146.1–937.4)[a]	959.6 ± 73.6 (146.1–1889.3)	↓
$CD4^+CD45RO^+$	1112.1 ± 272.2 (806.9–1655.1)	1120.8 ± 80.5 (211.6–2474.3)	
$CD8^+CD45RO^+$	1060.7 ± 217.9 (785.4–1490.9)	738.4 ± 62.2 (196.8–1651.7)	↑
$CD4^+CD45RA^+CD45RO^+$	32.4 ± 10.1 (16.2–50.9)[a]	7.8 ± 1.9 (0.0–50.9)	↑
$CD8^+CD45RA^+CD45RO^+$	71.2 ± 34.3 (18.2–135.5)[a]	19.7 ± 5.2 (0.7–135.5)	
COS and AM			
$CD3^+CD4^+CD28^+$	1209.6 ± 493.5 (670.6–2195.1)	1689.6 ± 100.0 (670.6–3230.0)	↓
$CD3^+CD8^+CD28^+$	340.6 ± 161.0 (47.6–602.6)	787.7 ± 67.8 (47.6–2041.2)	↓
$CD3^+CD4^+CD95^+$	1171.3 ± 333.8 (710.0–1819.8)	1138.8 ± 64.6 (636.9–2201.4)	↑
$CD3^+CD8^+CD95^+$	1203.4 ± 332.4 (745.0–1849.5)	1069.5 ± 62.2 (552.0–1965.6)	↑
$CD3^+CD4^+CD28^+CD95^+$	856.1 ± 313.2 (492.8–1479.6)	1009.9 ± 61.1 (492.8–2161.5)	↑
$CD3^+CD8^+CD28^+CD95^+$	284.4 ± 127.4 (45.5–480.6)	515.7 ± 43.7 (45.5–1278.0)	↑
$CD56^{dim}CD95^+$	72.9 ± 0.6 (72.3–73.5)[a]	48.3 ± 2.0 (30.3–73.5)	
$CD56^{bright}CD95^+$	81.4 ± 0.6 (80.8–82.0)[a]	45.5 ± 2.6 (22.2–82.0)	
$CD56^{dim}CD28^+$	28.5 ± 8.9 (19.6–37.4)	13.6 ± 2.1 (1.5–44.7)	
$CD56^{bright}CD28^+$	52.1 ± 3.9 (48.1–56.0)[a]	16.6 ± 3.3 (1.9–72.7)	
$CD56^{dim}CD95^+CD28^+$	25.8 ± 6.6 (19.2–32.3)	10.0 ± 1.4 (0.5–32.3)	
$CD56^{bright}CD95^+CD28^+$	51.1 ± 4.9 (46.2–56.0)[a]	14.8 ± 2.9 (1.7–56.0)	
T Lymphocytes			
$CD3^+CD4^+$ $CD25^+$	102.4 ± 21.6 (61.1–133.9)	143.3 ± 11.1 (8.0–388.8)	NE ?80–200
$CD3^+CD8^+$ $CD25^+$	56.6 ± 23.1 (30.2–102.6)	48.9 ± 6.7 (3.8–186.2)	NE
$CD3^+CD4^+$ $HLA\text{-}DR^+$	400.6 ± 149.4 (203.8–693.6)	186.8 ± 19.1 (32.8–693.6)	↑ ?50–150
$CD3^+CD8^+$ $HLA\text{-}DR^+$	249.1 ± 185.3 (63.6–619.8)	202.0 ± 28.1 (14.2–754.9)	↑
$CD3^+CD4^+$ $CD25^+$ HLA-DR	45.0 ± 14.1 (26.8–72.6	33.3 ± 2.9 (3.5–75.9)	↑
$CD3^+CD8^+$ $CD25^+$ HLA-DR	9.2 ± 5.9 (2.1–20.8)	9.9 ± 2.2 (1.3–71.7)	↑
$CD3^+CD4^+$ $CD69^+$	175.9 ± 60.6 (94.4–294.3)	131.9 ± 10.0 (50.3–297.5)	NE
$CD3^+CD8^+$ $CD69^+$	139.6 ± 15.9 (112.3–167.4)	125.6 ± 7.7 (57.1–247.2)	NE
Natural killer cell subtypes			
$CD56^+$	129.1 ± 17.7 (111.5–146.8)	123.4 ± 16.8 (36.7–345.4)	↑ 400–500; 160–400
$CD56^{dim}$	6.3 ± 1.4 (5.0–7.7)	5.7 ± 0.6 (1.6–14.6)	↑
$CD56^{bright}$	0.9 ± 0.0 (0.8–0.9)	1.0 ± 0.2 (0.3–7.3)	↓ 15–16
$CD56^{dim}CD25^+$	14.9 ± 11.9 (3.1–26.8)	6.4 ± 2.0 (0.4–45.9)	NE
$CD56^{bright}CD25^+$	17.1 ± 4.2 (12.9–21.3)	10.0 ± 1.5 (0.9–31.6)	↔
$CD56^{dim}HLA\text{-}DR^+$	16.4 ± 7.8 (8.6–24.2)	10.6 ± 2.1 (0.7–63.6)	↑
$CD56^{bright}HLA\text{-}DR^+$	17.0 ± 4.5 (12.5–21.4)	11.5 ± 1.2 (2.8–25.4)	↑
$CD56^{dim}CD25^+HLA\text{-}DR$	10.0 ± 8.8 (1.2–18.8)	2.8 ± 0.9 (0.3–18.8)	↑
$CD56^{bright}CD25^+HLA\text{-}DR$	9.4 ± .6 (8.8–10.0)	6.9 ± 1.3 (0.5–20.3)	↑
$CD56^{dim}CD69^+$	9.4 ± 1.2 (8.2–10.6)	6.3 ± 0.7 (1.6–14.9)	↓
$CD56^{bright}CD69^+$	0.9 ± 0.0 (0.9–0.9)	0.9 ± 0.1 (0.1–2.4)	↔
NKCA (% lysis)			
5:1 ratio	20.2 ± 14.0 (6.2–34.2)	15.6 ± 2.7 (1.1–65.3)	
10:1 ratio	39.3 ± 19.7 (19.6–59.0)	33.2 ± 4.1 (3.7–87.9)	
20:1 ratio	60.0 ± 18.3 (41.7–78.3)	53.8 ± 4.9 (5.3–117.2)	
40:1 ratio	67.4 ± 11.6 (55.8–79.1)	63.8 ± 4.1 (16.3–120.6)	
LPR (delta cpm)			
PHA	14.538,1 ± 773.2 (13764.9–15311.3)[a]	29.711.5 ± 3098.8 (4247.9–65073.0)	
OKT3	13.986,2 ± 126.6 (13859.6–14112.7)[a]	23.152,9 ± 2600.6 (5902.1–77928.3)	

Data are presented as cells·mm^3 (unless for CD4:CD8 ratio).
[a]Statistically different from non-IRP group; NE: no available evidences; COS: costimulatory molecules; AM: apoptotic markers; NKCA: natural killer cell cytotoxic activity; LPR: lymphoproliferative response; PHA: phytohemaglutinin.

Conclusions were essentially similar on progressively eliminating non-significant beta coefficients from these equations.

4. Discussion

Our data offer a substantial selection of normative values for lymphocyte subsets in sedentary but otherwise healthy older individuals. The findings do not seem greatly different from those for young adults, with the exception of low values for NK cell counts. Despite the limited age range of our data, the immune parameters showed some age-related changes within our sample; in particular, the $CD8^+$ naïve and memory cells, $CD3^+$ and $CD4^+$ cell activation, and relative values for $CD56^{dim}$ cells counts all increased with age. The consensus of other authors notes that over the full adult

Table 3
Statistically significant univariate regression analyzes of immunological parameters as a function of age, showing values at or close to statistical significance.

	R	R^2	Adjusted R^{2a}	β	SE	P
Absolute immunological values						
$CD8^+CD45RA^+$	.32	.10	.08	31.82	16.09	.056
$CD8^+CD45RA^+CD45RO^+$	.39	.15	.12	2.71	1.19	.029
$CD3^+CD25^+HLA\text{-}DR^+$	.32	.10	.08	1.00	.50	.054
$CD4^+CD69^+$	.37	.14	.11	4.88	2.05	.023
Relative immunological values						
$CD56^{dim}$	.35	.12	.09	13.64	6.81	.055

[a] NOTE: When a small sample is involved, the R square value in the sample tends to be a rather optimistic overestimation of the true value in the population (see Tabachnick and Fidell, 2001, p. 147), and the adjusted R^2 provides a better estimate of the true population value.

Table 4
Univariate correlations of immunological parameters with aerobic power and muscle strength.

Aerobic power (mL kg^{-1} min^{-1})		Muscle strength (N)		Entire group	
<22.6	≥22.6	<750	≥750	Muscle strength	Aerobic power
$CD56^{dim}CD25^+$	$CD4^+CD45RA^+$	–	$CD3^+HLA\text{-}DR^+$	$CD3^+HLA\text{-}DR^+$	$CD4^+CD69^+$
ABS: $r = 0.86$, $P = .003$	–	–	ABS: $r = 0.62$, $P = .006$	ABS: $r = 0.48$, $P = 0.004$	–
–	REL: $r = 0.-0.45$, $P = .048$	–	REL: $r = 0.64$, $P = .004$	REL: $r = 0.35$, $P = .039$	REL: $r = -0.35$, $P = .031$
$CD56^{dim}HLA\text{-}DR^+$	$CD3^+CD69^+$	–	$CD3^+CD25^+HLA\text{-}DR^+$	$CD3^+CD25^+HLA\text{-}DR^+$	–
ABS: $r = 0.92$, $P = .0001$	–	–	ABS: $r = 0.49$, $P = .041$	ABS: $r = 0.39$, $P = .020$	–
REL: $r = 0.91$, $P = .001$	REL: $r = -0.53$, $P = .016$	–	REL: $r = 0.48$, $P = .045$	REL: $r = 0.35$, $P = .039$	–
$CD56^{dim}CD25^+HLA\text{-}DR^+$	–	–	$CD4^+CD25^+HLA\text{-}DR^+$	$CD3^+CD4^+CD8^+$	–
ABS: $r = 0.82$, $P = .013$	–	–	ABS: $r = 0.48$, $P = .038$	ABS: $r = -0.35$, $P = .036$	–
–	–	–	REL: $r = 0.46$, $P = .046$	REL: $r = -0.34$, $P = .044$	–
$CD4^+CD45RO^+$	–	–	$CD8^+CD45RA^+CD45RO^+$	$CD8^+CD45RA^+CD45RO^+$	–
–	–	–	ABS: $r = 0.60$, $P = .019$	ABS: $r = 0.50$, $P = .005$	–
REL: $r = -0.57$, $P = .027$	–	–	REL: $r = 0.57$, $P = .026$	REL: $r = 0.37$, $P = .041$	–

ABS: absolute values (cells·mm^3); REL: relative values (%).

range, aging is associated with a decline in T cell function (Ginaldi et al., 1999; Makinodan et al., 1991; Pawelec et al., 2002), with decreased pools of naive T and B cells (Utsuyama et al., 1992), increases in the number of memory and effector T and B cells (Linton et al., 1987), an accumulation of late differentiated effector T cells, and a diminished B cell production of immunoglobulins, probably secondary to a reduced activity of T helper lymphocytes (Ben Yehuda and Weksler, 1992; Antonaci et al., 1987). An age-related up-regulation of $HLA\text{-}DR^+$ and $CD25^+$ (activation marker) on $CD3^+$ lymphocytes has also been described in older subjects (Rea et al., 1999).

Early reports suggested that NK cell numbers and activity were unchanged with aging (Fiatarone et al., 1989), but more recent investigators have generally described an increase in the proportion of $CD56^{dim}$ (mature) NK cells, a decrease in the number and/or activity of NK cells, with a decreased affinity for target cells (Grubeck-Loebenstein et al., 2009; Nasrullah and Mazzeo, 1992; Miyaji et al., 1997; Ruvakina et al., 1998), possibly accentuated in unfit subjects (Ross et al., 2004). The increase in the proportion of mature NK cells may contribute to the decline of NK cell function and thus the increased risk of infections and mortality in elderly individuals (Solana and Mariani, 2000). The numbers of both $CD56^{bright}CD16^+$ and $CD56^{dim}CD16^+$ mature subsets seem to be stable or even increased in older individuals, whereas the $CD56^{bright}CD16^-$ precursor subset is decreased (Beziat et al., 2011; Chidrawar et al., 2006; Le Garff-Tavernier et al., 2010). A decline in the number of $CD56^{bright}$ NK cells in particular may impair immune regulation, as this cell population plays a central role in cytokine secretion during the innate immune response (Simpson, 2011).

It remains uncertain how far adverse changes in immune function can be reversed by an increase of physical activity, although the limited relationships we have found between immune parameters and either aerobic power or muscle strength suggest that the variations of fitness seen in a healthy but non-athletic elderly population have at most a limited impact upon immune function. Simpson (2011) suggested that regular exercise might conserve immune function by forcing T cells into the circulation, encouraging the apoptosis of memory T cells, and thus making "space" for a release of further naive T cells. However, the demargination of cells from reservoir sites would probably require a higher intensity of exercise than that attained by most sedentary elderly individuals when they engage in a bout of exercise. Previous cross-sectional comparisons and short-term training studies of the elderly generally support our viewpoint. Shinkai et al. (1998) saw little difference in $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$ or $CD19^+$ counts between aerobically active and inactive elderly non-smokers; very fit individuals showed a superior T cell proliferative response to both PHA, and pokeweed mitogen, but the mixed lymphocyte reaction was not enhanced, making it unlikely that their T cell effector function was enhanced. Likewise, Arai et al. (2006) found that in elderly men the proliferative response to PHA was enhanced by aerobic training. Nieman et al. (1993) also made a cross-sectional comparison between fit and unfit women aged 67–85 years; the highly trained individuals had a 54% advantage of lytic activity and a 56% greater T cell proliferative response to PHA, but there were no inter-group differences in lymphocyte subset counts, and a 12-week training programme did not enhance either T cell function or resting NK cell activity in the sedentary group. Woods et al. (1999) also found no significant increase of NK activity with six months of aerobic training in elderly men. A dissident report from Crist et al. (1989) noted a 33% increase in resting NK cell activity in seven elderly subjects following 16 weeks of aerobic training. There is even less evidence of a positive response of immune parameters to resistance training (Raso et al., 2007; Flynn et al., 1999; Kapasi et al., 2003), although McFarlin et al. (2004)

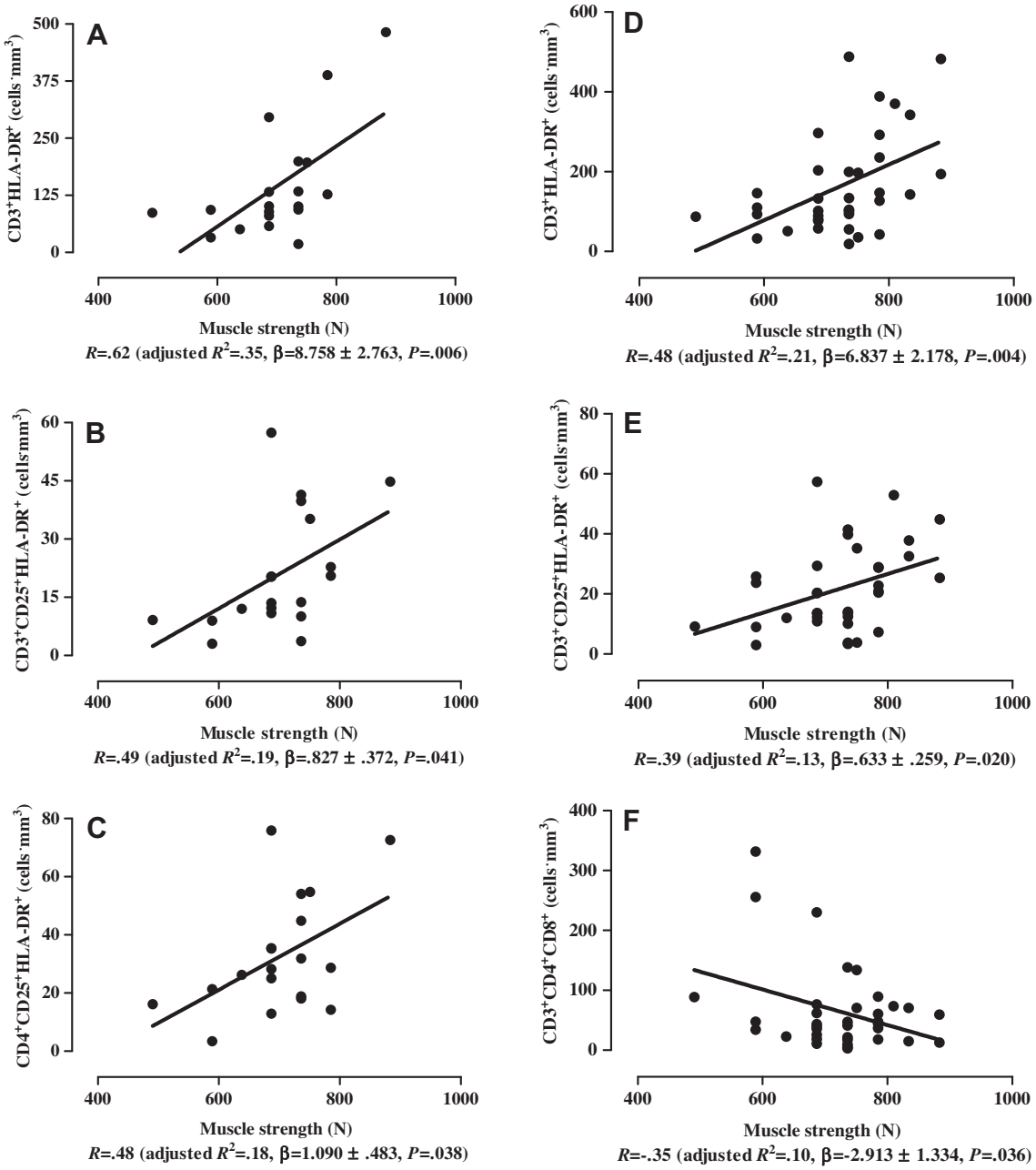

Fig. 1. Scatterplot for immunological phenotypic parameters according to muscle strength for percentile 50–100 (A–C) and total sample (D–F).

did observe some increase of NK cell activity. In reviewing available reports, Bruunsgaard and Pedersen (2000) concluded that physical training programmes acceptable to an elderly population are unable to bring about any major restoration of the senescent immune system.

Our observations have been based on fitness scores, rather than questionnaire estimates of habitual physical activity. Although fitness scores reflect both the genetic characteristics of an individual and his or her habitual physical activity, this approach to classifying the activity habits of the elderly avoids the problems of recent memory often encountered when using questionnaires in such populations. Our subjects were typical of most elderly people, not athletic and relatively unfit compared with some previously reported groups; as might be predicted from previous reports on the general elderly population, there was little evidence that lymphocyte counts and sub-sets, the proportion of naive and memory cells, NK cell sub-sets, co-stimulatory molecules, apoptotic markers and activation markers differed between the upper and lower halves of the fitness spectrum, whether this was assessed in terms of aerobic power or muscle strength. Furthermore, three subjects who were immunologically "at risk" had similar levels of fitness to the remainder of our subjects. In evaluating these last observations, it is important to note that only a very small number of our subjects fell into the "at risk" category, and for the purpose of this initial analysis assignment was based simply on a low $CD4^+$ count and a CD4:CD8 ratio <1.0, although currently, a cluster of biomarkers is recommended for a precise assessment of risk (Simpson and Guy, 2010).

A few statistically significant relationships were observed when we calculated univariate correlations between immune parameters and fitness measures; in particular, low levels of aerobic power were associated with low counts of $CD56^{dim}$ cells, and individuals

Table 5
Beta (significance level) of values from multiple regression analyzes of immunological parameters as a function of fitness score, depression, fatigue, quality of life (QOL), and carbohydrate intake (CHO) regardless of age. The dark shading indicates significant beta coefficients.

	FITscore	Depression	Fatigue	QOL	CHO
$CD56^{dim}$ (REL)	.236 (.158)	−.382 (.085)	.791 (.001)**	−.117 (.558)	−.003 (.982)
$CD3^+CD8^+$ (REL)	−.154 (.390)	.695 (.006)**	.156 (.461)	.625 (.008)**	−.064 (.702)
$CD4^+CD45RO^+$ (REL)	−.124 (.500)	.348 (.157)	.478 (.036)*	.466 (.048)*	.266 (.133)
$CD8^+CD45RA^+CD45RO^+$ (REL)	.466 (.013)*	.299 (.197)	.151 (.461)	.199 (.354)	.076 (.639)
$CD3^+$HLA-DR^+ (REL)	.210 (.210)	.357 (.108)	.339 (.090)	.016 (.938)	.290 (.071)
$CD3^+$HLA-DR^+ (ABS)	.497 (.001)**	.240 (.183)	.299 (.071)	r = .102 (.539)	.263 (.048)*
$CD3^+CD25^+$HLA-DR^+ (ABS)	.546 (.004)**	−.513 (.031)*	.195 (.342)	−.505 (.024)*	.004 (.978)
$CD8^+CD25^+$HLA-DR^+ (ABS)	−.032 (.864)	−.365 (.141)	.011 (.959)	−.739 (.003)**	.026 (.883)
Phytohemaglutinin	.388 (.057)	.260 (.308)	−.690 (.007)**	−.646 (.014)*	−.085 (.638)

*$P < 0.05$; **$P < 0.01$ (after Bonferroni correction); FITscore: fitness score calculated as Z values of muscle strength plus Z values of aerobic power divided by two; ABS: absolute values (cells·mm^3); REL: relative values (%).

Table 6a
Beta (significance level) of values from multiple regression analyzes of immunological parameters as a function of aerobic power, depression, fatigue, quality of life (QOL), and carbohydrate intake (CHO) regardless of age. The shading indicates significant beta coefficients.

	Aerobic power	Depression	Fatigue	QOL	CHO
$CD3^+CD16^+CD56^+$	.123 (.620)	−.458 (.114)	.594 (.040)	.009 (.971)	.039 (.870)
NKCA 5:1	.255 (.371)	.242 (.454)	.192 (.542)	.133 (.644)	−.242 (.378)
NKCA 10:1	.247 (.366)	.112 (.716)	.446 (.147)	.124 (.651)	−.226 (.387)
NKCA 20:1	.279 (.300)	.055 (.855)	.451 (.136)	−.007 (.978)	−.262 (.311)
NKCA 40:1	.330 (.228)	−.117 (.700)	.452 (.140)	−.015 (.956)	−.188 (.467)
$CD56^{dim}$	.263 (.357)	−.344 (.290)	.347 (.274)	−.032 (.911)	−.098 (.719)
$CD3^+CD4^+$	−.005 (.982)	−.305 (.255)	.187 (.469)	−.364 (.132)	−.046 (.835)
$CD3^+CD8^+$	.058 (.780)	.364 (.135)	.299 (.204)	.467 (.036)	−.263 (.198)
$CD4^+CD45RA^+$	−.320 (.190)	−.185 (.500)	−.136 (.609)	−.275 (.266)	.074 (.749)
$CD4^+CD45RO^+$	.018 (.935)	.123 (.624)	.443 (.079)	.199 (.378)	.036 (.866)
$CD8^+CD45RA^+$	−.010 (.969)	−.211 (.443)	.180 (.502)	−.105 (.669)	−.158 (.497)
$CD8^+CD45RO^+$	−.132 (.574)	.095 (.720)	.305 (.245)	.158 (.507)	.312 (.172)
$CD3^+$HLA-DR^+	−.251 (.174)	.444 (.039)	.205 (.310)	.088 (.631)	.460 (.013)
Phytohemaglutinin	.017 (.946)	.374 (.036)	−.679 (.023)	−.484 (.069)	−.039 (.017)

Table 6b
Beta (significance level) of values from multiple regression analyzes of immunological parameters as a function of muscle strength, depression, fatigue, quality of life (QOL), and carbohydrate intake (CHO) regardless of age. The shading indicates significant beta coefficients.

	Muscle strength	Depression	Fatigue	QOL	CHO
$CD3^+CD16^+CD56^+$	.199 (.342)	−.486 (.091)	.538 (.042)	−.076 (.768)	.092 (.644)
NKCA 5:1	.142 (.562)	.257 (.435)	.086 (.773)	.061 (.842)	−.119 (.612)
NKCA 10:1	.095 (.684)	.138 (.661)	.345 (.234)	.071 (.809)	−.105 (.639)
NKCA 20:1	.065 (.779)	.096 (.757)	.338 (.239)	−.051 (.860)	−.123 (.582)
NKCA 40:1	.065 (.783)	−.066 (.837)	.319 (.277)	−.062 (.834)	−.024 (.916)
$CD56^{dim}$	.223 (.359)	−.349 (.284)	.235 (.423)	−.136 (.651)	.025 (.912)
$CD3^+CD4^+$	.067 (.740)	−.324 (.237)	.187 (.448)	−.390 (.132)	−.052 (.787)
$CD3^+CD8^+$	.065 (.721)	.358 (.151)	.274 (.223)	.438 (.064)	−.237 (.183)
$CD4^+CD45RA^+$	.125 (.563)	−.287 (.324)	−.014 (.958)	−.302 (.268)	−.095 (.646)
$CD4^+CD45RO^+$	.085 (.658)	.104 (.685)	.432 (.072)	.165 (.493)	.041 (.823)
$CD8^+CD45RA^+$	.087 (.672)	−.237 (.390)	.181 (.469)	−.138 (.591)	−.167 (.397)
$CD8^+CD45RO^+$	.163 (.411)	.023 (.932)	.351 (.151)	.103 (.676)	.237 (.216)
$CD3^+$HLA-DR^+	.506 (.0005)	.253 (.138)	.288 (.066)	−.092 (.556)	.309 (.015)
Phytohemaglutinin	.288 (.158)	.299 (.266)	−.695 (.010)	−.597 (.026)	−.044 (.816)

with greater muscle force showed higher scores for several T cell activation markers. It is possible that the lack of relationships between aerobic fitness and T-cell subsets could be due to the limited range of fitness levels within our sample (although such a range is typical of the general elderly population). A further potential issue is the phenotyping methods that we used, since there have been recent reports of an inverse association between aerobic power and 'senescent/exhausted' $CD8^+$ T-cells, regardless of age and body mass index, when a four-color cytometric flow analysis system is employed (Spielmann et al., 2011). However, when other psychobiological variables (depression, fatigue and quality of life) were introduced into multivariate equations, these latter variables accounted for most of the variance in immune parameters. Proponents of psychoneuroimmunology have long argued the importance of personal well-being to effective immune function (LaPerriere et al., 1994). In part as a consequence of our initial selection, our subjects had relatively normal scores for depression, fatigue and quality of life. Thus, even larger effects might be anticipated across the full spectrum of older individuals. One complication in parceling out effects is that those with clinically significant depression, stressful life events and/or a poor quality of life would likely show an associated reduction of physical activity (Yosiuchi et al., 2006, 2007). However, the range of fitness levels observed in our sample showed little association with mood state or quality

Table 6c
Beta (significance level) of values from multiple regression analyzes of immunological parameters as a function of FITscore plus depression, depression, fatigue, quality of life (QOL), and carbohydrate intake (CHO) regardless of age. The shading indicates significant beta coefficients.

	FITscore	Depression	Fatigue	QOL	CHO
$CD3^+CD16^+CD56^+$	.065 (.843)	−.488 (.228)	.542 (.045)	−.014 (.959)	.090 (.670)
NKCA 5:1	.525 (.158)	−.158 (.717)	.070 (.803)	−.001 (.998)	−.203 (.384)
NKCA 10:1	.389 (.279)	−.173 (.686)	.334 (.236)	.021 (.939)	−.168 (.460)
NKCA 20:1	.242 (.500)	−.096 (.824)	.331 (.244)	−.080 (.779)	−.162 (.481)
NKCA 40:1	.347 (.342)	−.348 (.428)	.308 (.282)	−.114 (.692)	−.081 (.725)
$CD56^{dim}$	.246 (.518)	−.501 (.280)	.233 (.432)	−.104 (.730)	−.007 (.978)
$CD3^+CD4^+$	.218 (.506)	−.494 (.218)	.181 (.480)	−.412 (.123)	−.087 (.678)
$CD3^+CD8^+$	.080 (.787)	.307 (.396)	.273 (.247)	.445 (.070)	−.247 (.201)
$CD4^+CD45RA^+$	−.013 (.971)	−.242 (.572)	−.009 (.974)	−.251 (.377)	−.087 (.701)
$CD4^+CD45RO^+$	.150 (.632)	−.002 (.995)	.430 (.088)	.164 (.509)	.019 (.923)
$CD8^+CD45RA^+$	−.009 (.979)	−.206 (.611)	.184 (.484)	−.102 (.702)	−.162 (.453)
$CD8^+CD45RO^+$	.385 (.229)	−.266(.486)	.342 (.173)	.081 (.747)	.178 (.379)
$CD3^+HLA\text{-}DR^+$	.573 (.022)	−.105 (.713)	.283 (.133)	−.023 (.904)	.234 (.130)
Phytohemaglutinin	.660 (.029)	−.194 (.567)	−.711 (.005)	−.632 (.011)	−.145 (.421)

FITscore: (Z aerobic power plus Z muscle strength) divided by two.

of life, and our observations suggest that immuno-senescence may be countered more effectively by addressing psychological health than by engaging in moderate aerobic or resistance training.

We should finally underline that all of our observations were made on circulating blood. Blood concentrations of lymphocytes are probably the most important factor in gauging immune health, although since some 99% of these lymphocytes are located elsewhere in the body, altered cell numbers in the aging could reflect in part a redistribution of cells rather than alterations in absolute cell numbers. Our observations also refer to a selected group of elderly women who were fairly sedentary but in relatively good health, and findings might be very different in a broader sample of the elderly, including both those active to the level of Masters competition and those with more overt issues of mood state and quality of life. Although we deliberately excluded those with overt chronic infections, we must underline that we did not undertake exhaustive screening for chronic viral infections, a factor that can have a substantial impact upon the immune profile of the elderly. Further study is also needed to examine how far the associations with psychobiological variables are causal, how easily psychological health can be enhanced, and whether this will indeed have a favourable impact upon immune function.

5. Conclusions

Although elderly women show some univariate correlations between fitness markers and such characteristics of an aging immune system as alterations in T cell activation markers, memory cell counts, and $CD56^+$ cell counts, stronger correlations are seen relative to psychobiologic variables (depression, fatigue and quality of life). Longitudinal studies are recommended to examine how far the adverse psychological concomitants of aging can be reversed, and whether this may offer a helpful approach to the treatment of immuno-senescence.

Acknowledgements

This study was supported by a Grant from Sao Paulo Research Foundation (FAPESP) (2001/14976-2).

References

American College of Sports Medicine. General principles of exercise prescription. In: Franklin BA.(ed.), ACSM's Guidelines for Exercise Testing and Prescription (7th Ed.), Philadelphia, 2006.

Antonaci, S., Jirillo, E., Bonomo, L., 1987. Immunoregulation in aging. Diagn. Clin. Immunol. 5, 55–61.

Arai, M.H., Duarte, J.S., Natale, V.M., 2006. The effects of long-term endurance training on the immune and endocrine systems of elderly men: the role of cytokines and anabolic hormones. Immun. Ageing 3, 9.

Ben Yehuda, A., Weksler, M.E., 1992. Immune senescence-mechanisms and clinical implications. Cancer Invest. 10, 525–531.

Beziat, V., Duffy, D., Quoc, S.N., et al., 2011. Cellular immunology and immune regulation: $CD56^{bright}CD16^+$ NK Cells: A functional intermediate stage of NK cell differentiation. J. Immunol. 186, 6753–6761.

Bruunsgaard, H., Pedersen, B.K., 2000. Effects of exercise on the immune system in the elderly population. Immunol. Cell Biol. 78, 523–531.

Crist, D.M., Mackinnon, L., Thompson, R.F., et al., 1989. Physical exercise increases natural killer cell mediated tumor cytotoxicity in elderly women. Gerontology 35, 66–71.

Chidrawar SM, Khan N, Tracey-Chan YL et al. Ageing is associated with a decline in peripheral blood CD56bright NK cells. Immunol. Aging 2006; November 29. doi: 10.1186/1742-4933-3-10.

Fiatarone, M.A., Morley, J.E., Bloom, E.T., et al., 1989. The effect of exercise on natural killer cell activity in young and old subjects. J. Gerontol. 44, M37–M45.

Flynn, M.G., Fahlman, M., Braun, W.A., et al., 1999. Effects of resistance training on selected indexes of immune function in elderly women. J. Appl. Physiol. 86, 1905–1913.

Ginaldi, L., De Martinis, M., D'Ostilio, A., et al., 1999. The immune system in the elderly: II. Specific cellular immunity. Immunol. Res. 20, 109–115.

Grubeck-Loebenstein, B., Della Bella, S., Iorio, M., 2009. Immunosenescence and vaccine failure in the elderly. Aging. Clin. Exp. Res. 21, 201–209.

Kapasi, Z.F., Ouslander, J.G., Schnelle, J.F., Kutner, M., Fahey, J.L., 2003. Effects of an exercise intervention on immunologic parameters in frail elderly nursing home residents. J. Gerontol. Med. Sci. 58, 636–643.

LaPerriere, A., Ironson, G., Antoni, M.H., et al., 1994. Exercise and psychoneuroimmunology. Med. Sci. Sports Exerc. 26, 182–190.

Le Garff-Tavernier, M., Béziat, V., Decocq, J., et al., 2010. Human NK cells display major phenotypic and functional changes over the life span. Aging Cell 9, 527–535.

Linton, P.J., Haynes, L., Tsui, L., et al., 1987. From naive to effector: alterations with aging. Immunol. Rev. 160, 9–18.

Makinodan T, Blloom ET, James SJ et al. Immunity and aging. In: MSJ Pathy, (ed.), Principles and practice of geriatric medicine, Chichester: John Wiley, 1991; pp. 3–12.

McFarlin, B.K., Flynn, M.G., Campbell, W.W., et al., 2004. TLR4 is lower in resistance-trained older women and related to inflammatory cytokines. Med. Sci. Sports Exerc. 36, 1876–1883.

McNair DM, Droppleman LF. Manual for the profile of mood states. San Diego, CA: Educational and Industrial Testing Service; 1971.

Miyaji, C.H., Watanabe, H., Minagawa, M., et al., 1997. Numerical and functional characteristics of lymphocyte subsets in centenarians. J. Clin. Immunol. 17, 420–429.

Nasrullah, I., Mazzeo, R.S., 1992. Age-related immuno-senescence in Fischer-344 rats: influence of exercise training. J. Appl. Physiol. 73, 1932–1938.

Nieman, D.C., Henson, D.A., Gisewitch, G., et al., 1993. Physical activity and immune function in elderly women. Med. Sci. Sports Exerc. 25, 823–831.

Pawelec, G., Barnett, Y., Forsey, R., et al., 2002. T cells and aging, January 2002 update. Front Biosci. 7, d1056–d1183.

Raso, V., Benard, G., DA Silva Duarte, A.J., 2007. Effect of resistance training on immunological parameters of healthy elderly women. Med. Sci. Sports Exerc. 39, 2152–2159.

Rea, I.M., McNerlan, S.E., Alexander, H.D., 1999. CD69, CD25, and HLA-DR activation antigen expression on $CD3^+$ lymphocytes and relationship to serum TNF-alpha, IFN-gamma, and sIL-2R levels in aging. Exp. Gerontol. 34, 79–93.

Ross, Kristine M.; Keylock, K. Todd; Lowder, Thomas; McAuley, Edward; Woods, Jeffery A. Natural killer cell activity in older adults of varying fitness levels. Med. Sci. Sports Exerc. 2004; 36: S151.

Ruvakina, D., Laskain, G., Rubesa, G., et al., 1998. Age-related decline of perforin expression in human cytotoxic T lymphocytes and natural killer cells. Blood 92, 2410–2420.

Shephard, R.J., 1997. Physical activity, training and the immune response. Cooper Publishing, Carmel, IN.

Shinkai, S., Konishi, M., Shephard, R.J., 1998. Aging and immune response to exercise. Can. J. Physiol. Pharmacol. 76, 562–572.

Simpson, R.J., 2011. Aging, persistent viral infectionsand immunosenescence. Can exercise ''make space''? Exerc. Sport Sci. Rev. 39, 23–33.

Skevington, S.M., Lofty, M., O'Connell, K.A., 2004. The World Health Organization's WHOQOL-Bref quality of life assessment: psychometric properties and results of the international field trial. A report from the WHOQOL group. Qual. Life Res. 13, 299–310.

Solana, R., Mariani, E., 2000. NK and NK/T cells in human senescence. Vaccine 18, 1613–1620.

Strindhall, J., Nilsson, B.-O., Löfgren, S., Ernerudh, J., Pawelec, G., Johansson, B., Wikby, A., 2007. No immune risk profile among individuals who reach 100 years of age: Findings from the Swedish NONA immune longitudinal study. Experim. Gerontol. 42, 753–761.

Tabachnick B.G., Fidell, L.S., Using multivariate statistics, 4th Ed. Boston: Allyn & Bacon, 2001.

Targonski, P.V., Jacobson, R.M., Poland, G.A., 2007. Immunosenescence role and measurement in influenza vaccine response among the elderly. Vaccine 25, 3066–3069.

Utsuyama, M., Hirokawa, C., Kurashima, C., et al., 1992. Differential age-change in the numbers of CD4+, CD45RA+, and CD4+CD29+T cell subsets in human peripheral blood. Mech. Ageing Dev. 63, 57–68.

Woods, J.A., Ceddia, M.A., Wolters, B.W., et al., 1999. Effects of 6 months of moderate aerobic exercise training on immune function in the elderly. Mech. Ageing Dev. 109, 1–19.

Simpson, R.J., Guy, K., 2010. Coupling aging immunity with a sedentary lifestyle: has the damage already been done? – A mini-review. Gerontology 56, 449–458.

Spielmann, G., McFarlin, B.K., O'Connor, D.P., et al., 2011. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav. Immun. 25, 1521–1529.

Yosiuchi, K., Nakahara, R., Kumano, H., et al., 2006. Yearlong physical activity and depressive symptoms in older Japanese adults: Cross-sectional data from the Nakanojo study. Am. J. Ger. Psychiatr. 14, 621–624.

Yoshiuchi, K., Cook, D.B., Ohashi, K., Kumano, H., Kuboki, T., Yamamoto, Y.B.H., 2007. A real-time assessment of the effect of exercise in chronic fatigue syndrome. Physiol. Behav. 92, 963–968.